WHAT WE SHOULD KNOW ABOUT MALARIAL DISEASE.

WILLIAM KRAUSS, M.D.
(Professor of Pathology and Tropical Medicine, College of Physicians and Surgeons, Memphis, Tenn.)

Malarial disease is an infection[*] of the red blood corpuscles with one or more species of a hematozoon officially known as the *plasmodium.* Three species are known: *p. vivax,* which causes the "benign" *tertian* fever with its modifications; *p. malariae,* which, maturing in 72 hours, produces (when in single broods) *quartan* paroxysms (plasmodium malariae is the parasite of *quartan fever only* and the name should not be in a generic sense); *p. precox* occasions the various types of fever grouped under the head of *estivo-autumnal* by Marchiafava and Celli (*piccoli plasmodi*). Canalis as early as 1889 called attention to two types, one maturing in 24 hours, the other in 36-48 hours. Grassi and Feletti, and Mannaberg, and, later, in this country, Craig, insist upon a differentiation of species for these types, but it is more likely that they are merely differences in strain or environment. For instance, the *p. immaculatum* segments before it has reached half the size of the containing red blood corpuscle, resulting in at most 6 or 8 segments, and before an appreciable amount of melanin could have been metabolized; its crescents, developing more slowly, always have the characteristic ring of pigment. The pigmented quotidian *p. precox* is so generally associated with the unpigmented that it would be more than a coincidence to have this double infection so constant. The *malignant, tropical* or *estivo-autumnal tertian, Laverania* (order of crescents, Grassi and Feletti), matures in about 48 hours, but the broods group so diffusely that clinically it has been recognized as the parasite of the irregular fevers. The clinical types are the malignant tertian, remittent and continued fevers. All estivo-autumnal parasites have crescents as "resting" forms, and the official name of the species for all three is *p. precox.*

CLINICAL TYPES. Single groups of *vivax* produce fever paroxysms lasting approximately 11 hours, *according to the temperature chart, not the statement of the patient,* about every 48 hours, usually beginning in the forenoon. Thus, a chill may come at 10 a. m. on Monday and another at from 9 to 11 on Wednesday. Two groups, maturing on alternate days, give us quotidian paroxysms, and we say we have *quotidian tertian,* or *double tertian;* the time of appearance of the alternate paroxysms need not correspond to the time of the primary group. Thus, we may have a chill at 10 a. m. on Monday, one at 1 p. m. on Tuesday, one at 10 on Wednesday, one at 1 p. m. on Thursday, etc. If the two groups are only half a day apart we can have two paroxysms on Monday, none on Tuesday, two on Wednesday, etc. This is *double quotidian tertian. Triple tertian* gives us two paroxysms Monday, one on Tuesday, etc. Two or three cases of *quadruple tertian* are on record, but it is more usual for multiple groups to mature diffusely, giving us tertian remittent and continued fever; rarely, we have symptoms of perniciousness due to this parasite. The word tertian is used both for periodicity and species; hence it should be qualified, e. g., benign tertian, malignant or tropical tertian; the former is due to the tertian parasite (*p. vivax*), the latter to the estivo-autumnal parasite (*p. precox, var. Laverania*).

Single groups of the *quartan* parasite (*p. malariae*) produce 10-11 hour paroxysms about every 72 hours. Two groups give, for example, a paroxysm on Monday, one on Tuesday, none on Wednesday, one each on Thursday and Friday. This is *double* quartan. Three groups fill in all the days, hence we speak of it as *quotidian* quartan; it is most generally designated as *triple* quartan. Infection with the quartan parasite may give rise to indefinite clinical types. It rarely goes into the resting stage and may persist for years. Quartan infection is most difficult to eradicate, although yielding readily (symptomatically) to small doses of quinine. If in double quartan we have the history only of the paroxysms on Tuesday and Thursday we may think we are dealing with the tertian parasite—or if we see the case on Friday we may be deceived into believing that we are dealing with a tertian which has just become "double"; or, the alternate group may have only just become massive enough to begin to show on Thursday. Thus, in a variety of ways we can be deceived by a quartan infection. The diagnosis is important because it calls for more persistent treatment; it can only be made by examining the blood. I have a number of times fallen heir to cases of quartan infection because the first physician allowed too many relapses to occur.

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[*] For animal parasites the term "infestation" should be used. It is as yet not generally applied to blood parasites, only to intestinal.

To recapitulate, the estivo-autumnal parasite gives us quotidian paroxysms by maturing precociously; double malignant tertian cannot cause quotidian fever, because the duration of the paroxysm is so long that two groups give us remittent fever, the rise due to the second group intercepting the fall of the first. The quotidian estivo-autumnal fever, then, is due to single groups of parasites having a 24-hour cycle; quotidian tertian is due to two groups of the benign tertian parasite (having a 48-hour cycle) maturing on alternate days; quotidian quartan fever is due to three groups of (72-hour cycle) quartan parasites, maturing 24 hours (or nearly) apart. Tertian fevers, as we have seen, may be caused by any of the three species, typical tertian intermittents being in the vast majority of cases due to p. vivax. Malignant tertian, from the p. precox (48-hour type) is characterized by a longer duration of the fever, a lower and broken fever apex, and rarely exists in the pure form, since the groups tend to brood diffusely, some maturing in 36 hours, others not under 50 hours; this finds clinical expression in remittent fever, with perhaps an indistinct second and rarely a third chill; it is most often confounded with typhoid fever. The tongue, the facies, the belly, the complexion, the leaky skin at times, the restlessness and pains, ought to put the clinician on his guard and not let his malarial patient get into a "typhoid" state or lapse into perniciousness. In either case there is apt to be diarrhea, gurgling, or even hemorrhage. Pernicious fever may come from all three species. Thus we see that, except when we have frank, single-group intermittents, we cannot undertake to differentiate species from clinical symptoms.

A few words as to perniciousness. This is most often due to 24-hour types of estivo-autumnal parasites. In the peripheral blood these appear only as small, very delicate, symmetric rings. Others of the brood may have become crescents; extremely rarely we may find segmenting bodies, from which we can differentiate types; six to eight segments speak for the quotidian, whilst malignant tertian may divide into as many as thirty (I have one preparation showing 28 segments). Brood-containing corpuscles are apt to become impacted in capillary vessels, producing emboli, which

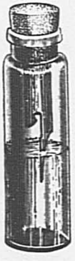

Figure I.

in turn filter back great numbers in localized areas, nearly always in certain organs or groups of organs. According to localization we have cerebral (comatose, etc.), pulmonic, gastralgic, choleraic, neuritic, diaphoretic and other types of pernicious fever. Hemorrhage is but an incident and does not justify a separate type. Likewise, hemoglobinuria is due to death of plasmodia en masse with liberation of endocellular hemolysin, is a remote event, does not attack new-comers, is not necessarily embolic, and has no place in the group of perniciosae. Pernicious fever usually develops gradually but it is not very rare to have a pernicious first paroxysm.

Latent and larvate malaria. Since the introduction of precision in blood examination one hears very little of larvate malaria. Cases of periodic neuralgia, urticaria, and a great variety of periodic symptom-groups are now probably less often ascribed to malarial infection than is really the case. Those who examine blood probably err on the other side, whilst those who are in the habit of treating malaria symptomatically may treat frontal sinusitis, etc., for malarial neuralgia. As to latent malaria, it is unfortunately still the practice to ascribe almost every kind of chronic ailment to "a touch of malaria." I have had cases referred to me as "malaria in the system" that had reached the point of incurability and whose real condition should have been long ago recognized, viz: visceral malignancy, gall bladder disease, abscess of the liver, miliary tuberculosis, latent sepsis, dysentery, uncinariasis, Banti's disease, malignant endocarditis, syphilitic fever, etc.

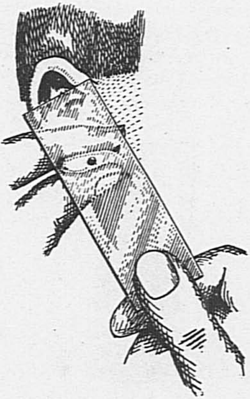

Figure II.

Persons who have been residents of malarious districts may acquire a relative immunity and harbor malaria parasites without having symptoms or at least without having fever. It is quite common for such persons to have "resting bodies," which are the gamete or sexual forms of the beginning of the long or mosquito cycle. Only persons harboring this phase of the organism can transmit the infection to mosquitoes, and this is most often the case with those up and walking about. Such persons are a menace to the community, and they may infect mosquitoes in their own environment, and become re-inoculated with active malaria. Such an event occurring in a refugee into anopheles territory may wonder where his fever came from, since he fancies he has just run away from it, yet he has actually started a focus. This responsibility of a malaria-infected individual should be explained to him, and the physician himself should never forget to be alive to his duty in such cases and insist upon having the patient remain under treatment. This is the more important since resting bodies are absolutely refractory to quinine and their appearance must be prevented.

Diagnosis. This is said to be accomplished by the clinical symptoms, the temperature chart, and the examination of the blood. For ideal work it is not only necessary to recognize the parasites but also the species, since the treatment and prognosis then become more precise. We have already alluded to the fallacies of the clinical symptoms. The therapeutic test, especially in cases of sepsis, is worse than useless. Latent malaria may become active when some condition lowering resistance, such as shock, bad hygiene, injury, the puerperium, and surgical operations, intervenes. This is the source of our drinking water malaria, watermelon malaria, etc. Now, if a puerpera has a chill, followed by fever, and quinine is given, either its oxytocic power or accident may cause the expulsion of a septic clot, and a case of "malaria" has been cured. The matter is more complex if a pus focus evacuates after a dose of quinine and the fever falls. Here the fancied security of having aborted an attack of malaria may lead to serious complications. We see it stated as axiomatic that leucocytosis is positive evidence against malaria. This is wrong. Malarial leucocytosis as a rule is low but exceptionally, especially the vaso-motor kind of pernicious and hemoglobinuric forms, may be very high. The *relative* increase of large mononuclears here becomes diagnostic in case of failure to find the parasites. In the presence of a leucocytosis, the increase in large mononuclears only becomes apparent if we make both absolute and differential counts, and then calculate the absolute number of each variety. It is then possible to tell malarial variation even if only four or five per cent. of the leucocytes are mononuclears. Whilst the normal number is about 300 per cm. (which rarely quite doubles during a septic leucocytosis) we find in malaria, in any stage, as many as 800 to 1,000 per cm. Then, too, there is the quinin leucocytosis, coal tar and bath leucocytosis, which is so generally overlooked. The normal fasting blood rarely contains over 6,500 leucocytes. If we take the sample with due regard for the conditions normally influencing leucocyte movement we can get far more information from the study of the leucocytes than is ordinarily the case.

Whilst clinical symptoms and quinin test are not to be relegated entirely, especially by those with no great experience in malaria blood work, it is quite the practice to persist in the exhibition of toxic doses of quinin long after the astute clinician should be aware that he is dealing with some other fever. On the other hand, certain types of malaria are incredibly refractory to quinin—sometimes because the criterion used is "cinchonism," which in some persons develops after very small doses or very little absorption. With the addition of suitable correctives larger doses can be borne.

No one should attempt to examine blood for malaria unless he has studiously practiced some approved method and found it reliable and himself competent. Unfortunately, none of the ready-made stock stains can be depended upon. Many are faulty even before they are sent out, and all of them deteriorate sooner or later, and sometimes quite suddenly. The most important thing to learn about blood stains is to know when a film is improperly stained and to be able to differentiate faults of technic from those of deteriorated stain. All the one-solution stains, whether Goldhorn's, Leishman's, Wright's, Hastings', Giemsa's, etc., tend to lose titer and become acid, only the eosin taking hold. Another mistake commonly made is to use them all alike, whereas each author's method must be strictly followed with his own stain. After considerable experience in teaching students and practitioners I have evolved a method which comes nearer being automatic than any other I have ever read or heard of, and I have tried scores of them. The method

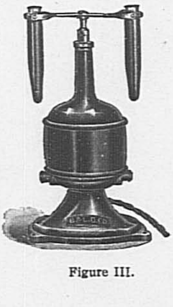

Figure III.

is more suitable for malaria than for leucocytes; even here it is superior for all except basophiles, which have no practical significance anyway. It should be added that neither Ehrlich's nor Jenner's stain have any place in malaria work. Of the one-solution stains I prefer a fresh home-made Goldhorn, Hastings or Wright in the order given; one and one-half grains of the powder is dissolved in an ounce of freshly redistilled methyl alcohol and the solution kept in a cork-stoppered bottle *and never removed except by pouring from the bottle.* Making up more than this is waste, as it will not keep.

THE AUTHOR'S METHOD.

Provide a cork-stoppered homeopathic vial containing absolute alcohol. Drive the butt end of a steel pen firmly into the under surface of the cork and break off one nib of the pen; when the bottle is corked, the pen point becomes sterilized. (Fig. 1.)

Next, have a supply of microscope slides, a 50 cc. bottle of absolute alcohol, a number of pieces of plain gauze, napkin size, as used in throat practice. Now proceed as follows:

1. Wash the hands and rub off with alcohol.
2. Prepare six slides by wiping them first with alcohol, then with dry gauze.
3. Seated on the right of the patient, facing in the opposite direction, grasp the lobule of the patient's right ear between the thumb and index finger of the left hand so as to cause turgescence; rub it briskly with a wisp of gauze dampened—not saturated—with alcohol.
4. Plunge the pen into the presenting end of the lobule with a quick stroke to the depth of 3 mm. (1/8 inch).
5. Squeeze very gently and wipe off the first drop—the next will flow more freely—let it collect in a small, globular, not flat, drop on the surface.
6. With the right hand pick up one of the slides, lower its distal end on the index finger of your left hand, as shown in the illustration (Fig. 2), then depress the proximal end of the slide until the apex of the drop is just touched, taking on no more than the size of a typewritten "O." Under no circumstances compress or blot the drop.
7. By pronating the right hand, the slide is tilted upside down toward the operator, taken into the left hand, the drop being now on the upper surface of the slide close to the grasping thumb.
8. With the right hand a second slide is now taken up, its lateral edge placed transversely to the surface of the first slide and proximally to the drop, tilted forward at an angle to suit the operator (about 45 degrees) and gently and very slowly swept across the blood, so as to distribute it over the surface, resting a moment *on* the drop to allow the blood to spread laterally.
9. Fan the filmed slide rapidly in the air until dry. The remainder of the slides are filmed in the same way, wiping off the bleeding point and extruding a fresh drop before each smear.

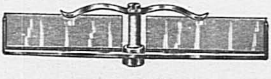

Figure IV.

It is best to practice on a willing victim, at first without drawing blood to get the drill, then with blood to learn how to get good spreads. It can also be practiced before a mirror with blood from one's own ear. The mastering of this technic is important because it enables one to send blood to the laboratory if not prepared to examine it. Scrupulous cleanliness, some deftness and speed, absence of dust and air currents and strict adherence to directions are necessary for success.

Flies will quickly eat off dried blood if allowed access.

Smearing with a needle, cigarette paper or by pushing the slide in advance of the blood or by holding it lengthwise or making the film on cover glasses need only to be mentioned as ways not to do it. The above method leaves all the elements undamaged on one film. It distributes the leucocytes ideally for differential counting; at the lateral fringe they are massed in proper proportion; at the terminal fringe the largest elements are collected, enabling one to judge marked relative increases of large mononuclear cells off-hand. Crescents and segmenting bodies also group here.

STAINING OF THE FILM: I.—EQUIPMENT FOR BLOOD WORK.

1. A table before a window facing north. to eliminate the direct rays of the sun.
2. A sink or washstand close by, over which is a rack for the stains.
3. A standard microscope with triple nosepiece, Abbe condenser, a good 2 mm. immersion lens with wide angle and good penetration, even if at expense of flatness of field, the usual 4 and 16 mm. dry objectives, a one-inch eye piece and mechanical stage.
4. A urine centrifuge with one graduated tube (Fig. 3) and Author's slide drying head which is exchangeable for the tube carrier (Fig. 4).
5. Several 30 cc. pipet bottles (Fig. 5). The ground "stopper" surface should be well paraffined.
6. Slides, coverglasses (square), forceps, test tubes, filter paper, litmus paper, distilled water, etc.
7. Two Pasteur-Petri dishes, 100 mm. diameter.
8. One gram Azur II. Gruebler, dry.
9. Ten grams eosin, water-soluble, yellowish, dry (Gruebler).
10. A one per cent. solution tannin containing a few drops of chloroform.
11. 500 cc. Merck's guaranteed methyl alcohol. Commercial wood alcohol redistilled over caustic potash will answer.
12. 100 cc. C. P. Aceton. This removes precipitates from stained slides and is useful for final washing of blood pipettes.

II.—METHOD.

Make up 25 cc. one per cent. solution of II. in distilled water and 25 cc. aqueous eosin solution, add a few drops chloroform to each and keep in dropping bottles; select two delivering the same number of drops per cc.

1. Flood blood slide with methyl alcohol for one minute, during which time it must be covered by the bottom half of a Petri dish to prevent evaporation. For this purpose it is convenient to have a slip of thick glass, smaller than the slide, cemented on a small square of window glass. The slide is laid on this, the alcohol poured on and the cover placed over it; in this way the draining off of the alcohol is minimized.
2. While the slide is "fixing," measure 7 cc. distilled water in the graduated centrifuge tube, drop in exactly four drops of the eosin solution, shake, drop in six drops of the azure, shake again, and pour into the lid of the Petri dish. Into this place the still wet slide (inverted), letting one end rest on the edge. The staining solution will just cover the blood smear; it is left on for exactly five minutes. Remove, rinse rapidly in a glass of water and immediately dry on the centrifuge.

The slide can now be examined dry under the low power to see if the nuclei of the white cells are stained very deeply. If this is not

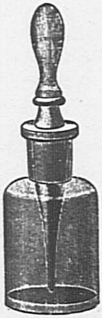

Figure V.

the case the amount of blue in the mixture was insufficient or the methyl alcohol was acid The correct quantity of blue can be judged by examining the mixture in the dish immediately after it is made over a white surface, when the mixture must be quite blue. If the nuclei have been found too pale, the film is stained as before, using one drop less eosin. Old films are apt to come up too blue; this can be reduced after drying by pouring on tannin solution and immediately rinsing off with water, and drying on the centrifuge. Precipitates can be removed by wiping the dry slide with a pledget of cotton dripping wet with pure aceton; it will exaporate at once. This cotton must never be used a second time, because it rapidly absorbs water.

Slides must never be dried with blotting paper or by heat nor allowed to dry spontaneously; in the absence of a centrifuge they must be waved in the air until dry.

A good field is now selected under a low power and a drop of immersion oil placed on it, then the film searched with the 2 mm. objective without a coverglass. If the slide is to be preserved, a coverglass is put on without balsam, using more oil if necessary. (Canada balsam is acid and soon bleaches the film.)

III.—THE APPEARANCE OF THE SPECIMEN.

The red cells are orange to pink, but if slightly grayish, it is a good fault. The lymphocytes have a partridge blue cytoplasm and a purple nucleus. The neutrophiles will be pale pink or heliotrope, showing purple neutrophile granules, the nuclei deep violet. The large mononuclear cells have a pale blue cytoplasm, a rather light-colored nucleus and a few deep purple perinuclear granules. The platelettes are heliotrope with deep purple nuclei; often one or more are found within a red cell and *mistaken for malaria parasites.* These latter *are never any color but pale blue,* the denser portions deep sky-blue; their chromatin is *carmin-red* and is the only element taking this color. By this means they can be positively identified. Unless the normal elements take on the colors specified we can not be sure of having stained the plasmodia. Just before the paroxysm and in the early hours of the fever it may take an hour to find a single one. After twelve hours they become quite numerous and are usually easily found. Quartans are found in every stage; estivo-autumnals brood diffusely and small rings are generally found if careful search is made, although most numerous during the remission.

IV.—APPEARANCE OF THE PLASMODIA.

The youngest forms of the parasite are differentiated by the beginner with difficulty. They appear of pale blue rings one-fourth the size of the containing cell and in the beginning may be at the edge; they enclose an achromatic zone, at one pole of which a minute dot of a carmin-red color can be seen. The quartan is the most compact and dense of the three, the estivo-autumnal the most delicate and pale. In the latter it soon divides and spreads around the ring, forming a horseshoe; this is absolutely characteristic. The tertian rapidly becomes varicose and filamentous; if the film has been dried rapidly, the most delicate lace-work results; at least one pigment granule appears within the thickened blue cytoplasm before the chromatin divides, which it does completely, into round granules, except when very filamentous. The quartan is deep blue in all stages, attains no growth under 18 hours, shows black, coarse pigment early, tends to spread across the cell in a broad ribbon, the chromatin divides late and slowly. The estivo-autumnal parasite retains its slender ring shape until it is quite large, forms pigment late and only slightly if at all, and which clumps early into one large block. The segmenting tertian makes over sixteen segments in a double row, the quartan eight to twelve in a single row, its pigment being now quite abundant, coarse and black, that of the tertian being more brown owing to its fineness and transparency.

The resting bodies can be differentiated early but the consideration of these would carry us too far; those of the benign species are spherical, those of the estivo-autumnal are sausage-shaped (usually called crescents). So-called flagella develop only after the blood has been shed for some time; this requires special technic. For ordinary observation they can be seen in fluid blood under a coverglass. The accompanying pictures will explain differences. The subjoined table will give the chief characteristics of the three species in parallel columns.

	P. VIVAX.	P. MALARIAE.	P. PRECOX.
Short cycle	48 hours	72 hours	20 to 50 hours.
Gametes	Spherical	Spherical, smaller, coarser pigment	Crescentic.
Young Parasite	Rather compact ring, achromatic center	Very compact, dark blue ring perfectly white center	Pale, open, delicate ring, hemoglobin shows through.
Chromatin	Does not stain w. methylen blue	Does not stain w. methylen blue	Stains dark blue w. methylen blue.
Chromatin divides	In granules *after* pigment appears. Sometimes forms ribbon	In granules *after several* pigment specks appear	Spreads laterally very early whilst ring is very small.
Pigment	Early, fine brown.	Early, coarse black	Late or none, soon clumps.
Pigment in fresh blood	Fine, actively dancing	Coarse, black, lazy	Very active at first.
Motility in fresh blood	Actively motile, filaments early	Slowly motile, remains compact	Actively motile, retains ring shape.
Appearance of fresh blood	Cell early enlarges and becomes pale	Cell contracts and becomes darker	Crenation, retraction of cytoplasm, brassy color.
Half-grown parasite	Many secondary loops, varicosities, much fine pigment	Very coarse, dark, varicose, ribbon forms, compact black, coarse pigment	Still, ring-shaped, signet form, slightly pigmented.
Color changes in stained blood	Schuffner's dots with Goldhorn's (red stippling)	Cell stains deeper	Basophile degeneration in deeply stained films (blue specks).
Segmenting	16-24 segments in 2 circles	8-12 segments in one circle	Rarely found; 6-30 segments.
Time of appearance	Most abundant after fever; scarce before chill and later	All stages	Generally only during growing stage (rings) but develop diffusely, hence may be found any time.

(TO BE CONTINUED)